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RADIOISOTOPE LOCALIZATION IN EXPERIMENTAL MEDIONECROSIS OF THE AORTA

GRANT L. CHRISTIAN

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RADIOISOTOPE LOCALIZATION IN EXPERIMENTAL MEDIONECROSIS OF THE AORTA

Grant L. Christian

Thesis submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF MEDICINE

Department of Surgery
Yale University School of Medicine

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Grant L. Christian

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CHAPTER I

INTRODUCTION

A degenerative process in the aortic media (1, 2, 3) referred to as "medionecrosis of the aorta" is generally accepted today as the underlying pathology of dissecting aneurysm of the aorta. Neither hypertension nor arteriosclerosis have been shown to cause medionecrosis (3, 4, 5, 6, 7, 8), and indeed similar structural changes of the medial connective tissue occur in pregnancy (2, 9), Marfan's syndrome (10, 11, 12), in dissecting aneurysms of unknown cause (13, 14, 15, 16, 17, 18), and in some aortas in which dissection has not occurred (19, 20). Certain other conditions such as scoliosis, infantile cortical hyperostosis, and thoracic cage deformities of unknown cause may represent a generalized defect in mucopolysaccharides, or other connective tissue substances, and indeed dissecting aneurysms have been observed to occur associated with these conditions (21).

The diagnosis of aortic medionecrosis is a histologic one, however, and cannot be made during life unless biopsy of the aorta be considered. Such a tissue diagnosis has been made, of course, from material taken when the aorta has been repaired, but this is only possible when dissecting aneurysm has occurred. This catastrophic complication of

medionecrosis therefore presents as the first indication of the presence of the vascular disease. Certain patients might be designated as having a high risk of medionecrosis (Marfan's Syndrome) but no technique has been available clinically to assess the state of the aorta which permits detection of the defect before dissecting aneurysm occurs.

In order to devise an in-vivo examination of the aortic media one would first like to demonstrate its feasibility in experimental animals. A model simulating medionecrosis of the aorta is available in lathyrism induced experimentally in young growing animals such as rats. The aortic media lesions produced in experimental lathyrism in rats are strikingly similar to those seen in histologic sections of human's aortas which have undergone dissection (21, 22).

The present study uses experimental lathyrism in the rat as a tool in studying the metabolism of sulfomucopolysaccharides in the aortic media. This analysis is undertaken with the use of radioactive S^{35} in an attempt to recognize any abnormalities of uptake in the media before death due to dissecting aneurysm. The value of early detection of aortic degeneration, by demonstrating abnormal accumulation of sulphur-containing mucopolysaccharides, at a time before dissection occurs will be discussed.

Human lathyrism, with its classical syndrome of muscle cramps and paraplegia, has been known for centuries to

be caused by the excessive ingestion of various types of the flowering sweet pea (23, 24, 25, 26, 27, 28, 29, 30), but it has only been in recent years that interest has developed in experimentally induced lathyrism. This disease has been produced in animals by the feeding (31, 32, 33, 34, 35, 36, 37), or by injecting daily small amounts (38, 39, 40), of beta-aminopropionitrile. It has also been produced by feeding a 50% diet of Lathyrus odoratus seeds (21, 41, 42, 43, 44, 45, 46, 47, 48). The lesions in the rat are more extensive than the human manifestations and include the following: paraplegia, demineralization and deformities of the spinal vertebrae and long bones of the body (41), weight loss, listlessness, and rumpled fur, altered collagen metabolism (31, 49, 50, 51, 40, 52), widening and disruption of the epiphyseal plate with altered metabolism of the mucopolysaccharides (53). The lesions produced in the aortic media include accumulation of acellular ground substance with disruption and fragmentation of the elastic laminae (41, 44, 45, 54), increased metachromasia with evidence of accumulation of acid mucopolysaccharides (44, 45, 47, 55), fibroblastic proliferation (46), thickening of the media (44), and dissecting aneurysms resulting in death (41, 42). These aortic lesions are identical to those that have frequently been described in human dissecting aneurysms (1, 21, 22).

Various theories have been proposed to explain the

etiology of the connective tissue defect in the aortic media and various other tissues in experimental lathyrism. Some of these theories include: (a) a failure of intramolecular cross linking between collagen fibers (51, 52, 56, 57), (b) a failure of complex formation between non-collagenous protein and chondroitin sulphate (31), and (c) altered metabolism of the mucopolysaccharides (21, 43, 44, 45, 58, 59, 60, 39). Most investigators agree that, while there may be defects in the collagen formation, one of the basic defects is with the acellular mucopolysaccharides composing the ground substance.

There are three reports in the literature of attempts to study the uptake of radioactive sulphur in the aorta of lathyritic animals (31, 36, 61). The first of these to be done was that of Ponseti, et al. (61), and involved the injection of 60 microcuries of S^{35} (as $Na_2S^{35}O_4$) I. P. into lathyritic rats with sacrifice 24 hours later and the preparation of radioautographs of the aorta and various other tissues. They found no greater uptake of S^{35} in the aortas of lathyritic animals when the radioautographs were grossly compared to those of normal controls. The second report was that of Kowalewski (36), who analyzed aortic tissue of lathyritic cockerels after injecting one microcurie of S^{35} per kg. of bird's weight and sacrificing 24 hours later. He reported no significant increase in the uptake of radioactive sulphur in the experimental animals. The first

of these investigators (61) found the typical aortic histological pattern involving fragmentation of elastic fibers, accumulation of acellular ground substance, thickening and micronecrosis of the media, and reported that from 35 to 50% of the experimental animals died from dissecting aneurysms. The second investigator did not study the aortic histology of the lathyritic animals but reported that 16% of the birds died of dissecting aneurysms.

The third report of S^{35} uptake in the aortic media of lathyritic animals was that of Kennedy and Kennedy (31), who found no significant abnormal uptake in rats. Of import to note in this study is that none of the animals demonstrated gross or microscopic changes in the aorta, and none of the animals died of dissecting aneurysms. For a more detailed and complete review of the literature pertaining to human dissecting aneurysm, see Appendix VI; and for experimental lathyrism, see Appendix VII.

In contrast to the three previous studies using S^{35}, the present writer has demonstrated a highly significant difference in isotope uptake and excretion by the lathyritic aorta, a difference which corresponds to the peculiar histologic staining characteristics of the diseased aorta, and which suggests for the first time that by isotopic technique diagnosis of aortic medio necrosis may be possible without aortic biopsy and before dissecting aneurysm occurs. This study differs from the previously reported work in five

important respects: (1) The observations were made over a protracted period. (2) Only radioactivity bound to the aortic tissue was measured. (3) The amount of isotope given each subject was controlled by weight. (4) An internal control in each subject was used, comparing two different tissues (xiphoid and aorta). (5) A more quantitative measure of radioactivity was used (grain counting).

CHAPTER II

METHOD

Twenty-seven albino male rats of Blue Spruce strain were fed a normal Purina chow and tap-water diet ad libitum until they were four weeks of age. At this time they were weighed and 13 of them were begun on a special lathyritic diet consisting of finely ground Purina chow thoroughly mixed with beta-aminopropionitrile fumarate (courtesy of Dr. Fost, Abbott Laboratories, North Chicago, Illinois), 0.18 per cent by weight. This method, described in Pontseti in 1959, will produce lathyrism in rats and a significant incidence of dissecting aneurysm of the aorta (37). The remaining animals were continued on their original diet. At the time the special diet was begun all the animals appeared healthy and active, with weights ranging from 45-75 grams. The animals were observed every day and permitted to take water and either of the two diets ad libitum. They were weighed weekly.

At the end of 21 days of the special diet, the weight of each animal was recorded to the nearest 10 grams and all were given $Na_2S^{35}O_4$, 2 microcuries per gram of body weight, by intraperitoneal injection. Two of the control group which were to serve as background for the isotope studies received no S^{35}. The animals were replaced in their original

cages and continued the two diets as before.

Two animals of each of the two diet groups were sacrificed by overdose with ether on each of the following days after the isotope was given: 1, 3, 7, 12, 18, 25. From each animal the aorta, xiphoid process, and a part of the left muscular hemidiaphragm were removed and fixed.

The fixation solution included the following: phosphomolybdic acid, ethanol, formaldehyde and water, as described by Pearse (62). All animals were X-rayed after death to demonstrate bone deformities. Tissue was removed from the fixative after 18 hours and transferred to 70 per cent alcohol. Sections of the arch of the aorta, the xiphoid, and the diaphragm were dehydrated as described (62) and imbedded in a single block of paraffin. Sections were cut to show the aorta as a ring. Section thickness varied from 4 to 7 microns, but any variation in slide thickness applied equally to all three tissues. Sections were mounted on slides precleaned with acid. Three slides were made from each animal. Paraffin was removed after mounting the slide by the usual methods involving zylene and alcohol rinses. Next the deparaffinized slides were immersed in 2 per cent Na_2SO_4 for three hours. This was done according to the method of Pearse (62) to remove any unbound radioactive S^{35} sulfate. This is a significant difference from the procedures of Ponseti (61), Kowalewski (36), and Kennedy and Kennedy (31) who had previously done radioautographic

studies and tissue analysis for S^{35}.

Following the Na_2SO_4 rinse the slides were rinsed in distilled water for fifteen minutes and stained with a combined elastic tissue and alcian green stain. Some tissues were stained, in addition, for hematoxylin and eosin. The alcian green stain is described by Hukill and Putt (63) as staining mucus and mucopolysaccharides green, elastic fibers black, nuclei blue to black, collagen red, and other elements yellow. It is further described as being excellent for illustrating the degenerative changes occurring in the media of large arteries such as are found in medial necrosis and aortitis due to syphilis. The elastic stain was used to better demonstrate the effects of lathyrism on the elastic laminae of the aortic media.

Following the staining procedures the slides were rinsed in distilled water and were immediately taken to the dark room where they were individually dipped in a prepared Kodak NTB bulk photographic emulsion. The technique of the dipping is critical and the following procedure was adhered to:

1. Kodak NTB bulk emulsion* was transported by air and refrigerated until ready for use.
2. All work was done in complete darkness.
3. The emulsion was melted in a small beaker immersed in a water bath kept at a constant

---

*Eastman Kodak Company, Rochester, New York.

40°C. in the dark room. The emulsion was allowed to stand for thirty minutes, thus allowing any air bubbles to rise. Following melting, the emulsion was poured into a small slide-shaped vial which was also kept suspended in the 40°C. water bath.

4. The slides were removed from the distilled water, shaken briefly, and touched on edge to a piece of absorbent paper to rid of excess fluid. They were then individually dipped in the emulsion vial with a slow steady movement and slowly withdrawn to allow a thin constant uniform coating.

5. The emulsion-coated slides were dried in complete darkness in a vertical drying rack for one hour, after which they were placed in small, clean, black, light-proof plastic boxes containing a small amount of drierite and silica gel to absorb any excess moisture.

6. Each box was sealed with black plastic electrical tape and stored in a refrigerator at a constant temperature of 40°C. for exactly six weeks.

After the six week's exposure, the slides were developed in the dark room in complete darkness by the following procedure:

1. Kodak D19 developer . . . . . .six minutes
2. Distilled water rinse . . . . . .briefly
3. Kodak acid fixer. . . . . .ten minutes
4. All above solutions were kept constant at 18° C.

Following fixation with the acid solution, the slides were rinsed in a pan of slowly moving cold tap water for forty-five minutes. They were then dehydrated and cover slips attached.

Grain Counting

Analysis of the radioactivity of each piece of tissue was performed by grain counting with a micrometer, as

described by Gross, et al., (64). Using the eyepiece micrometer, one-seventh of the oil-immersion-field was counted. Hereafter, "oil-immersion-field" refers, therefore, to 1/7 of the eyepiece field. A method of selecting the part of the tissue to be counted under low power was used because the photographic grains are not visible with the low power eyepiece. In this way, a possible bias was avoided because the oil immersion objective was substituted without shifting the position of the slide, and the grain counts performed in the corresponding part of the slide. Tissues from normal animals that did not receive the isotope were processed identically and concurrently and analyzed by the same counting technique to provide a value for the radioactive background.

Aorta

Four areas were selected from the ring of aortic tissue at 0^{o}, 90^{o}, 180^{o}, and 270^{o}. Because three slides were prepared from each animal, a total of 12 grain-count determinations were made upon the aorta, for each individual subject.

Xiphoid

Four separate areas were chosen for grain counting of the xiphoid tissue; one field at each end of the tissue, and a section about one-third the distance from each end were used. As in the aorta, these areas were selected under low power and the oil immersion objective substituted without

moving the slide.

Muscular Diaphragm

By a similar method, four separate areas were selected in the diaphragmatic tissue.

CHAPTER III

RESULTS

External Examination

The difference in size and weight amounting to almost 15 per cent between the experimental and control groups was first noted 12 days after starting the beta-aminopropionitrile diet. The experimental animals were smaller (refer to Figure 1, and Graph 1). In addition, the fur of the animals on the lathyritic diet became sparse and unkempt, and they appeared listless. These changes began at approximately the same time as the difference in weight was noted. The difference in weight became marked after the third and fourth weeks. One-third of the experimental animals developed partial paraplegia with dragging of the hind legs. A tabulation of weighings is presented in Appendix I.

The first bony deformity obvious to palpation was noted in the lathyritic group 19 days after the start of the diet as a moderate kyphosis in the thoracolumbar region.

Aortic Rupture

One animal was found dead 12 days after S^{35} injection. Autopsy revealed exostosis deformity of the sternum, kyphoscoliosis, massive hemothorax, and a longitudinal tear 4 mm in length in the lesser curvature of the aortic arch. (Figure 2.) No other animal died of spontaneous aortic

rupture under the conditions of the present experiment.

Bony Lesions

Whole-body radiographs made at the time of sacrifice and after removal of the aorta, xiphoid, and a portion of the muscular diaphragm, revealed moderate to severe deformity of the vertebrae in every case fed the lathyritic diet. No animal fed a normal diet exhibited these changes. In addition, the majority of the experimental animals revealed varying degrees of osteoporosis of the skeleton, most marked in the vertebrae, widening and displacement of the epiphyseal plate in the proximal femur, narrowing of the intervertebral disc space suggesting disc degeneration, and frequent new periosteal bone formation at sites of tendon and ligament insertion on the long bones (Figure 1).

Background Radioactivity

Two normal rats which did not receive S^{35} were used for determination of "background count." The same process of tissue seleciton, cutting, staining, application of the emulsion and developing was used as for the animals given the isotope. The emulsion exposure time was identical and concurrent and in the same light-proof boxes. The results were 1 to 9 grains per field (average 5.1) in the aorta, 1 to 8 (average 3.5) in the diaphragm, and 3 to 12 (average 6.9) in the xiphoid. Grain counts from these animals which received no isotope are summarized in Appendix V, and from

the animals in both diet groups which received isotope are summarized in Appendices II, III, and IV.

Aortic Media S^{35} Uptake

Table 1 summarizes grain counts of the aortic media and demonstrates the marked difference between the diet groups. These data are also presented in Graph 2. It is noted that in the normal diet group the radioactivity in the aorta reaches background between the 12th and 18th day, whereas in the lathyritic aorta at any point along this time-scale the radioactivity is 2 to 4 times greater and no overlap between the individually paired animals was observed. Extrapolation of the data suggests that the radioactivity of the aorta in the lathyritic group reaches background at about day 30. The difference between the two groups can be summarized as a much greater initial uptake of isotope and a proportionately longer disappearance time in the lathyritic animals. See Appendix II for complete data on grain counting in the aorta.

TABLE 1

AVERAGE AORTIC MEDIA GRAINS PER OIL-IMERSION-FIELD

Days Post S^{35} Injection	Experimental Animals	Normal Controls
1	320	98.5
3	158	78.1
7	200	38.8
12	111, 132*	30.4
18	90.4	7.7 (background levels)
25	35.3	10.8 (background levels)

Notes: *Died of dissecting aneurysm.

Each number, with the exception of the one designated with * represents an average of two animals, and has been calculated from 24 oil-immersion-fields. Note the constantly greater numbers in the experimental animals. Refer also to Graph 2.

Histology of Aorta

Histologic examination of the aorta prepared with alcian green and elastic stain was characterized by increased uptake of the alcian green, and uneven, irregular separation of elastic fibers in the lathyritic animals compared with normal controls (Figure 3). The increased staining with alcian green was uniform throughout the media and no "cystic pools" were observed. This greater staining with alcian green and the even distribution of the stain corresponded exactly with the distribution of photographic grains. No localized "hot"

areas could be identified. Increased staining with alcian green and increased radioactivity were somewhat higher in the inner two-thirds of the media than in the outer one-third. Adventitia stains hardly at all, nor does it contain more than background radioactivity. The intima of the rat aorta is too thin to be evaluated with regard to isotope uptake.

The animal that died of dissecting aneurysm had slightly more radioactivity in the aortic media than was found in two lathyritic animals that were sacrificed on the same day that this animal died spontaneously. The range of grain counts in these three subjects was within the same distribution. No significant difference could be demonstrated (see Table 1). Figures 4, 5, and 6 are representative sections of the aortic media demonstrating the precipitated photographic grains in both lathyritic and control animals.

Xiphoid Process S^{35} Uptake

Twenty-four hours after isotope injection the radioactivity in the xiphoid process was higher in the lathyritic animals than in the controls on a normal diet. However, at all subsequent time periods therewas no significant difference between the two diet groups. This is summarized in Table 2 and Graph 3, and suggests that the isotope was distributed in the xiphoid in a similar manner in both groups. The complete grain-count data is found in Appendix III.

TABLE 2

AVERAGE XIPHOID PROCESS GRAINS PER OIL-IMMERSION-FIELD

Days Post S^{35} Injection	Lathyritic Animals	Normal Controls
1	612	366
3	417	385
7	415	531
12	306	351
18	339	139
25	109	81

Notes: Observe the initial greater numbers of grains in the xiphoid of lathyritic animals on day one, but subsequently there is no such constant relationship. Compare with the curve of S^{35} uptake as demonstrated in Graph 3.

Muscular Diaphragm S^{35} Uptake

Twenty-four hours after isotope injection the average grain count in the muscular diaphragm was twice as great in the lathyritic diet group as in the normal diet control group, but only one-tenth that in the lathyritic aorta and one-twentieth of that in the xiphoid process. By the third day the isotope concentration in the diaphragmatic muscle was equal to background. See Appendix V for complete data.

Xiphoid to Aorta Ratio

Analysis of the data given above which indicate

TABLE 3

XIPHOID GRAINS PER OIL-IMMERSION-FIELD/AORTIC GRAINS PER OIL-IMMERSION-FIELD FOR LATHYRITIC AND NORMAL CONTROL ANIMALS

	Average X/A in each slide		Average X/A in each animal		Average X/A for each sacrifice day	
Day	Lathyritic	Normal	Lathyritic	Normal	Lathyritic	Normal
1	1.58	3.32				
	3.44	2.72	2.59	4.46		
	2.76	7.35				
	1.69	4.86			2.12	4.39
	1.70	5.10	1.65	4.32		
	1.58	3.02				
3	3.23	6.13				
	2.49	5.39	2.86	5.67		
	2.88	5.49				
	3.22	3.60			2.67	4.63
	1.76	3.82	2.49	3.59		
	2.50	3.49				
7	2.03					
	2.33		2.21			
	2.58					
	1.81	21.1			2.03	15.6
	1.94	13.5	1.86	15.6		
	1.83	12.4				
12	2.09	11.1				
	2.26	10.9	2.59	12.4		
	3.40	15.1				
	1.86	27.7			2.72	13.8
	2.64	7.87	2.84	15.2		
	4.01	9.95				
18	4.02	10.6				
	5.09	15.0	4.36	14.5		
	3.98	17.7				
	4.18	34.4	3.45	28.8	3.9	21.6
	3.69	26.7				
	2.58	24.8				
25	5.65	6.90				
	4.03	7.70	4.51	6.35		
	3.86	4.43				
	1.23	7.10			2.81	8.4
	0.65	9.33	1.11	10.4		
	1.44	14.7				

distribution of radioactivity similarly in the xiphoid process of both groups but of significantly higher concentration in the aorta was used to present in each animal a ratio which could serve as an "internal control" independent of the isotope dosage. (Note that the isotope was given on a weight basis and presumably was identical in all animals.) The comparison of xiphoid and aortic grain counts in each animal is designated the X/A ratio. This ratio is presented in summary in Table 3 and Graph 4. A significant difference between the diet groups was noted for every animal in this study. The differences are noted to be greatest during the period about two weeks after administration of the isotope (see Graph 4).

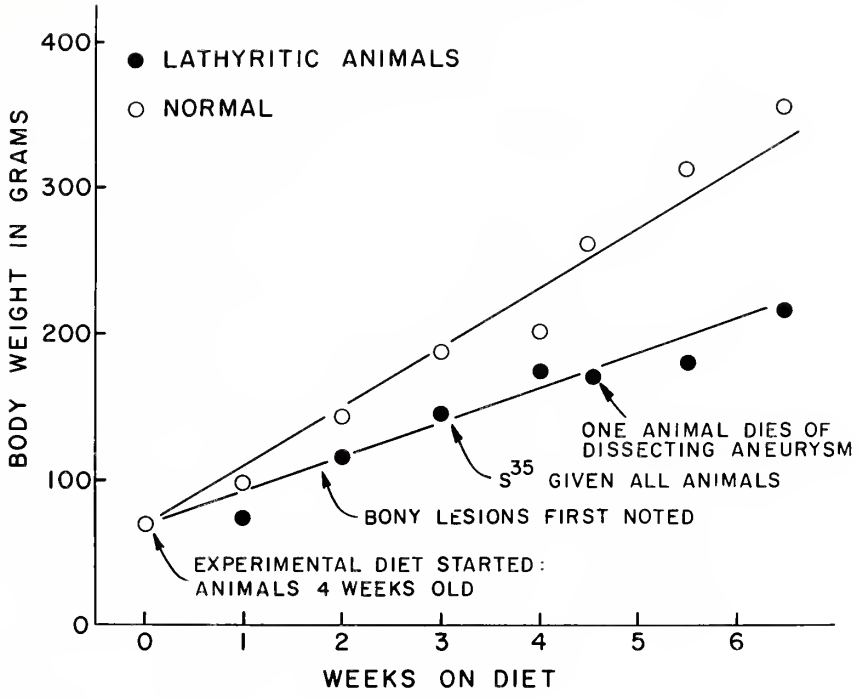
Comparison of Histologic and Isotopic Differences

Changes in the isotope content of the tissues studied paralleled changes in staining with alcian green. Table 4 summarizes these differences.

TABLE 4

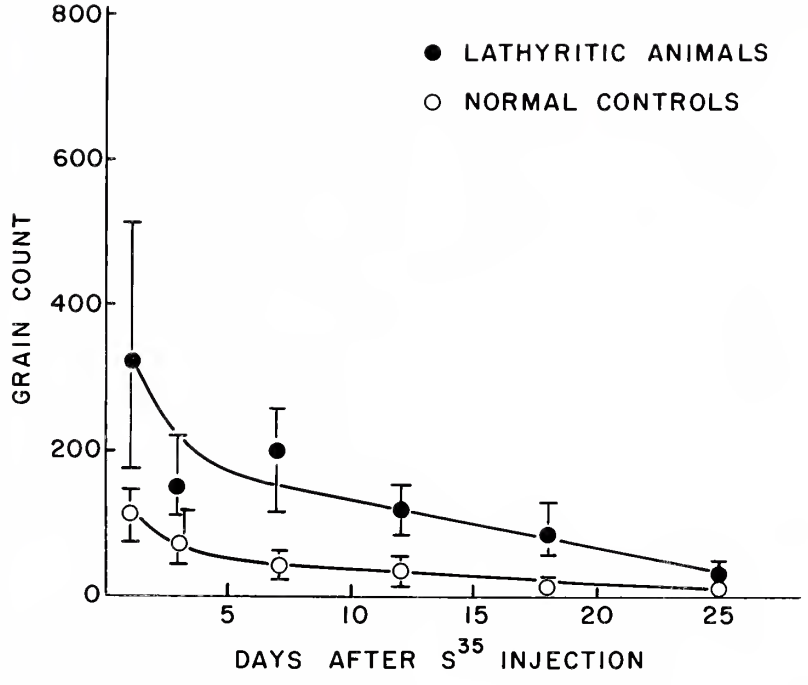
COMPARISON OF DEGREE OF ALCIAN GREEN STAIN WITH AMOUNT OF S^{35} ACTIVITY AS MEASURED BY GRAINS PER OIL-IMMERSION-FIELD

	Lathyritic Animals		Control Animals	
	Alcian Green	Grain Count	Alcian Green	Grain Count
Aortic Media	+++	+++	+	+
Aortic Adventitia	0	background	0	background
Xiphoid Process	+++++	+++++	+++++	+++++
Diaphragm	0	background	0	background



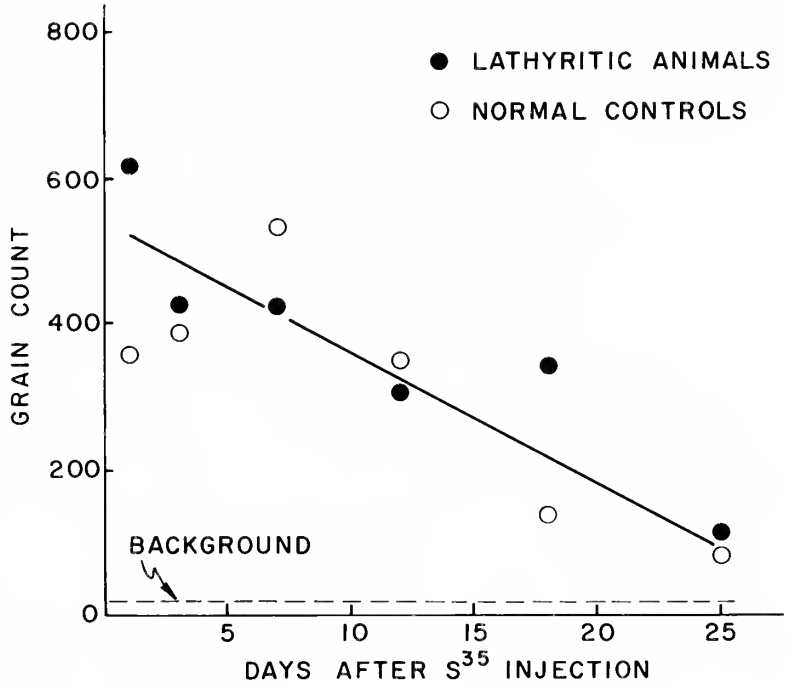
GRAPH 1

Weight gain in lathyritic and control animals. Average of body weights of all lathyritic and control animals measured weekly until S^{35} and thereafter at time of sacrifice.



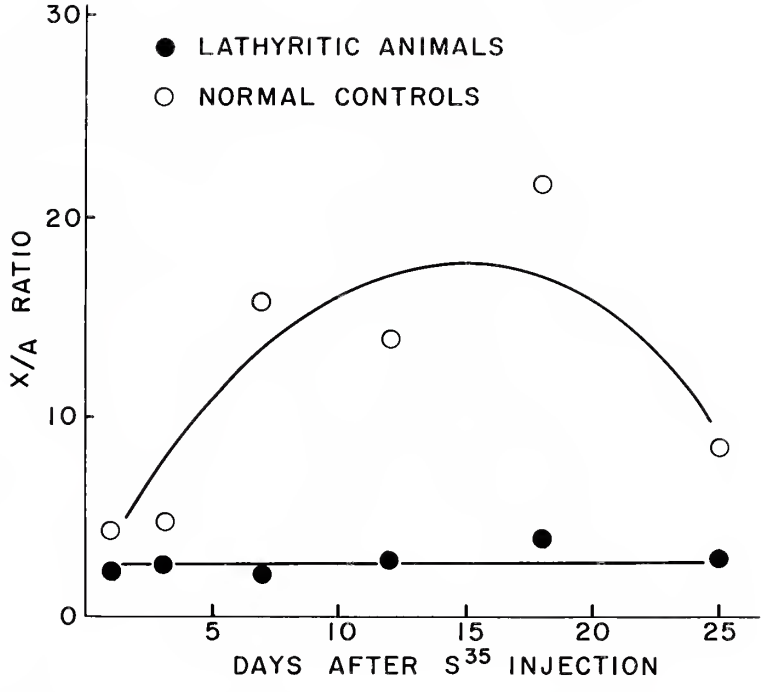
GRAPH 2

S^{35} disappearance from aorta. Average grain counts from aorta of lathyritic and control animals sacrificed in pairs at intervals after administration of S^{35}. All data corrected for isotope decay. All animals received an amount of isotope based upon body weights (see text -- methods). At all time intervals the lathyritic aorta contains significantly more radioactivity than the control aorta.



GRAPH 3

S^{35} disappearance from xiphoid. Average grain counts from xiphoid of lathyritic and control animals sacrificed in pairs at intervals after administration of S^{35}. All data corrected for isotope decay (see text -- methods). There is no significant variation in the data from the two diet groups.



GRAPH 4

Ratio of radioactivity in xiphoid to aorta in the lathyritic and control animals. The maximum difference between the groups is observed at about two weeks after S^{35} given.

FIGURE 1

Radiographs comparing normal control rat (bottom) and experimental lathyritic rat (top). Both animals 8 weeks of age; the lathyritic animal had been on beta-aminopropionitrile diet for 4 weeks. Note the general skeletal osteoporosis, disc degeneration, kyphosis, new bone formation of mid-femur, and overall smaller size of the lathyritic animal as compared to the normal.

FIGURE 2

Close-up photograph demonstrating dissecting aneurysm in lathyritic animal that died after 32 days on the beta-aminopropionitrile diet. The metal probe tip is resting just inside the tear in the lesser curvature of the aortic arch. Note the large blood clot to the lower right which had to be dissected away from arch to demonstrate the lesion.

FIGURE 3

Alcian green--elastic tissue stains of cross sections taken from aortic arch in normal control rat (top) and experimental rat (bottom) that have been on the beta-aminopropionitrile diet for 21 days before sacrifice. Note the markedly increased stain with alcian green, wide and irregular separation of elastic laminae, and lack of size uniformity of elastic fibers in the lathyritic media as compared to the normal.

FIGURE 4

Microphotographic comparison of precipitated photographic grains in aortic medias of control animal on top (animal No. 17) and lathyritic animal on bottom (animal No. 1). Both animals sacrificed 1 day post S^{35} injection. Note the greater number of grains per unit area and the more intense stain with alcian green in the lathyritic media. The elastic stain has been somewhat "washed out" by the emulsion developing process. 400X.

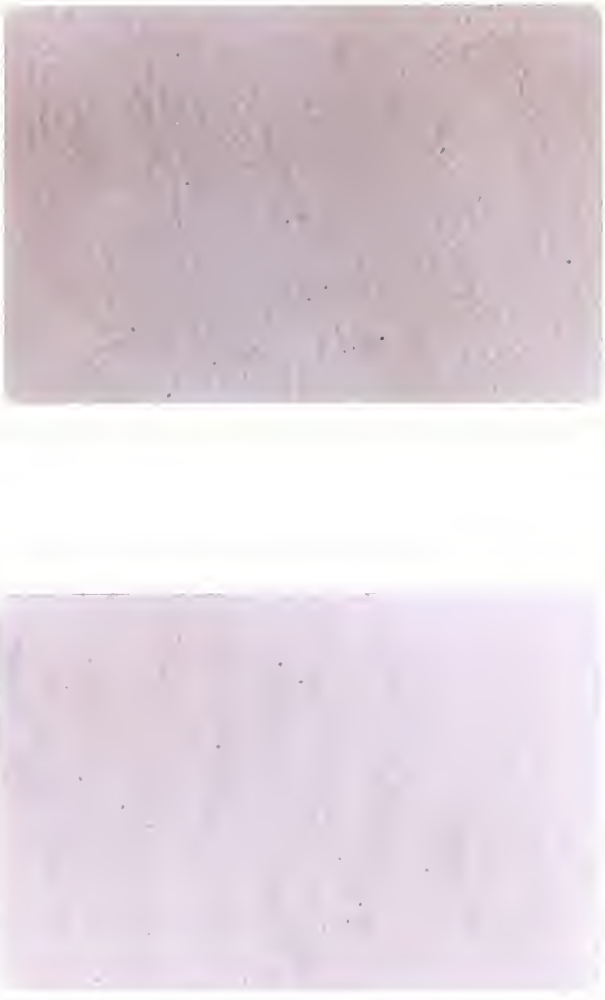


FIGURE 5

Microphotographic comparison of precipitated photographic grains in aortic medias of control animal on top (animal No. 20) and lathyritic animal on bottom (animal No. 4). Day 3 post S^{35} injection. Note the greater number of grains per unit area and the more intense stain with alcian green in the lathyritic media. Similar findings as in Figure 4, although there are fewer grains per unit area. 400X.

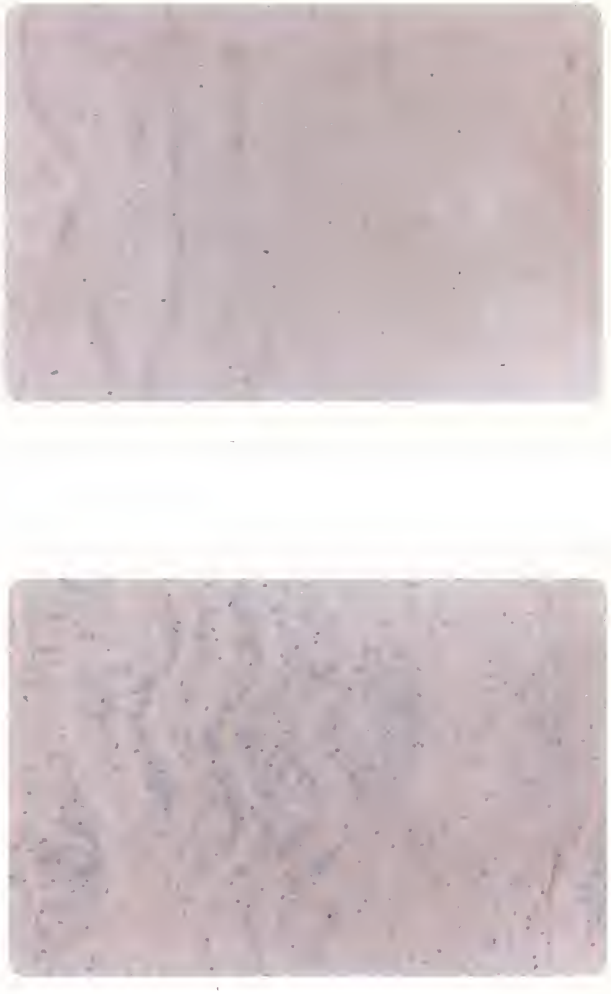


FIGURE 6

Microphotographic comparison of precipitated photographic grains in aortic medias of control animal on top (animal No. 22) and lathyritic animal on bottom (animal No. 5). Day 7 post S^{35} injection. Note the greater number of grains per unit area and the more intense stain with alcian green in the lathyritic media. Similar findings as in Figures 4 and 5. 400X.

CHAPTER IV

DISCUSSION

The methods and procedures used to induce experimental lathyrism during the present study produced the typical lesion, manifest by weight loss, changes in body fur, kyphoscoliosis, bony changes, paraplegia, and lesions in the aortic media. The histologic changes in the aortic media are identical to those previously described (21, 41, 42, 43), including thickening of the vessel wall, disruption and fragmentation of the elastic fibers, and increase in acellular ground substance. One animal died of dissecting aneurysm of the aorta. Other investigators (Ponseti and Baird, 41) have reported that if the diet is continued for a longer period than was used in this experiment, dissecting aneurysm of the aorta occurs in one-third of rats. The present data is not inconsistent with this. It has been previously demonstrated (38, 44, 45, 47, 55) that histologic stains for mucopolysaccharides demonstrate increased metachromasia in aortic media of lathyritic rats. Others have shown (65, 66, 67, 68) that S^{35} is taken up in high concentration in tissues that are rich in mucopolysaccharides. In the present study, the aorta of animals rendered lathyritic demonstrated greater alcian green staining and greater radioactivity than tissues taken from animals on a normal diet. Of the previous studies

in which S^{35} was given to animals made lathyritic, all (31, 36, 61) fail to demonstrate a greater concentration of the isotope in the diseased aorta than in the normal. The present study indicated a definitely greater concentration in the diseased aorta than in the normal. The techniques used in the present study differ from those previously reported, in that the isotope was given on a weight basis to all animals, the animals were studied for a period of 25 days after the isotope was given, radioactive sulphate not bound in the tissues was removed during the processing of the histologic slides, and analysis of the radioactivity was performed by counting individual precipitated photographic grains. The data in the present study indicate a significant difference in the aortic media of lathyritic animals compared with normals, not only in the histologic appearance and degree of alcian green stain, but also in the uptake and relief of S^{35}. It seems reasonable that the increased concentration of S^{35} is related to the fact that there is an accumulation of sulphur-containing material, presumably mucopolysaccharide and, in particular, chondroitin sulphate B. These data do not indicate whether depolymerization of the mucopolysaccharides, as suggested by others (42, 44), is characteristic of the lesion.

No localized "pools" of radioactivity were observed in the lathyritic aortic media. Indeed, the distribution of the isotope as indicated by the distribution of the grains in

the photographic emulsion was uniform throughout the aortic media, although somewhat higher in the inner two-thirds of the aorta. Histologic examination of the same tissue revealed accumulation of acellular ground substance separating and disrupting the elastic lamellae, but distributed diffusely throughout the aorta. The absence of pools of accumulated sulphur-continaing material which have been noted in human pathology may be one distinguishing factor between the experimental lesion in rats and the lesion occurring in the human patient.

Analysis of the precipitated photographic grains in the radioautographs of the xiphoid reveals no consistent difference in the amount of radioactivity between the lathyritic group and the normals. No previous study has been made of hyaline cartilage, of which the xiphoid is composed, although numerous other studies of metabolically active epiphyseal plate cartilage have been performed in the lathyritic animal (31, 32, 33, 34, 35, 41, 48, 61, 69, 70). Some of these studies have shown S^{35} uptake to be greater than normal, others less than normal, and some no change.

Comparison of radioactivity in the xiphoid with the aortic media was made to establish an "internal control" by which effective uptake of isotope in sulphur-contining tissues could be expressed as a constant ratio, regardless of the dose of isotope administered. The xiphoid to aorta ratio calculated from these data revealed in this study a distinct

difference between the diet groups for each slide, each animal and each period of observation (Table 3 and Graph 4). The average X/A index for the lathyritic animal at each time period is constantly lower than the corresponding index for the normal controls (Graph 4). Inasmuch as no difference in the isotope concentration in the xiphoid was demonstrated, the observed difference indicates that the difference is manifest in the aorta, rather than in the other sulphur-containing tissues.

Sulphur^{35} content of the aortic media in the single animal dying of dissecting aneurysm is only slightly greater than that measured in lathyritic partners of the same age. This is not considered a significant difference. Nevertheless, the possibility is not excluded that a significant difference might be noted if the experimental method were altered to continue the diet until one-third (37) or more animals had died of dissecting aneurysm while sacrificing an age-matched lathyritic animal for comparison with each animal that dies of aortic rupture.

These data suggest, although not for the first time, that a difference exists between the aorta in lathyrism and in normals which can be measured without biopsy or histologic techniques if isotopic methods are used. If this difference can be established in the human subject with aortic medial necrosis, it may permit a diagnostic approach using isotopic method which would indicate the aortic lesion before it is complicated by dissecting aneurysm.

CHAPTER V

SUMMARY

No method presently available permits diagnosis of aortic medial necrosis before it is complicated by dissecting aneurysm, although the histologic characteristics of the aorta under this circumstance was described 25 years ago and a similar lesion can be produced experimentally. A review of the pertinent literature of experimental lathyrism in rats is presented, and the similarity of the aortic lesion to the lesion in humans dying of dissecting aneurysm is reviewed. The accumulation of mucopolysaccharide, disruption and fragmentation of elastic lamellae, fewer elastic fibers, and increased thickness of the aortic wall were noted. The literature reveals (31, 36, 61) studies of S^{35} uptake in the lathyritic aorta in rats and cockerels. None of these reports indicated abnormal isotope uptake in the lathyritic aorta.

Sulphur^{35} ($Na_2S^{35}O_4$), two microcuries per gram of body weight, was injected into rats made lathyritic by feeding a diet containing 0.18 per cent beta-aminopropionitrile and an equal number of control animals fed a normal diet. Pairs of animals from each diet group were sacrificed at intervals following the injection. Tissue sections of the aortic media, xiphoid process and muscular diaphragm were prepared for

histology and radioautographs. Grain counts were performed on these sections with an eyepiece micrometer. A significantly greater uptake of isotope in the media of the lathyritic aorta than in the control was demonstrated by a ratio of 2:1 to 4:1. Isotope uptake in the xiphoid process was greater in the lathyritic animals on the first day after isotope injection, but thereafter was not significantly different between the diet groups. The uptake of isotope in the muscular diaphragm was slightly greater than background during the first three days, and the comparative low grain counts in this tissue reveals that soaking with Na_2SO_4 was successful in washing out the unbound sulphate. Comparison of radioactivity in the xiphoid with that of the aorta (X to A ratio) is another expression of the fact that differences in isotope uptake exist in the lathyritic aorta which provide a means of separating the two diet groups. These data suggest that with improved methods of isotopic diagnosis a method may be developed which will demonstrate the characteristic by which aortic medial necrosis can be recognized clinically before dissecting aneurysm occurs.

APPENDICES

APPENDIX I

ANIMAL WEIGHTS AND AMOUNT OF S^{35} INJECTED

Animal No.	Weight at S^{35} injection to nearest 10 grams	Weight at sacrifice to nearest 10 grams	Amount of S^{35} injected in micro-curies	Days after S^{35} injection at time of sacrifice	Total days on beta-aminopro-pionitrile diet
1	140	150	280	1	21
2	140	150	280	1	21
3	150	140	300	3	23
4	160	160	320	3	23
5	140	170	280	7	27
6	140	170	280	7	27
7	140	160	280	12	32
8	170	180	340	12	32
9	160	200	320	18	38
10	140	190	280	18	38
11	140	200	280	25	45
12	150	220	300	25	45
13*	150	150	300	12	32
17	180	190	360	1	--
18	190	200	380	1	--
19	190	190	380	3	--
20	190	190	380	3	--
21	180	190	360	7	--
22	200	210	400	7	--
23	170	280	340	12	--
24	200	250	400	12	--
25	200	320	400	18	--
26	200	300	400	18	--
27	200	330	400	25	--
28	190	350	380	25	--
32#	150	350	--	--	--
33#	140	360	--	--	--

*died of dissecting aneurysm

animals for radioactive background study

APPENDIX II - A

PRECIPITATED PHOTOGRAPHIC GRAINS PER OIL-IMMERSION-FIELD IN AORTIC MEDIAS OF LATHYRITIC ANIMALS. COMPARE TO FINDINGS OF NORMAL CONTROLS AS LISTED IN APPENDIX II - B.

Day Post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
1	1	1	188	268	252	192	230		
1	1	2	187	201	183	142	178	201	
1	1	3	182	258	171	172	196		320
1	2	4	356	422	387	384	386		
1	2	5	387	472	368	450	419	438	
1	2	6	520	511	499	508	508		
3	3	7	142	134	152	141	142		
3	3	8	97	111	93	129	107	118	
3	3	9	103	94	119	104	105		158
3	4	10	204	229	178	172	195		
3	4	11	189	190	240	229	212	198	
3	4	12	186	156	190	222	188		
7	5	13	222	258	213	222	229		
7	5	14	176	208	191	200	194	202	
7	5	15	211	240	165	120	184		200
7	6	16	265	197	206	237	226		
7	6	17	120	95	128	106	112	198	
7	6	18	259	264	231	281	259		

APPENDIX II -A --(CONTINUED)

Day Post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
12	7	19	91	110	113	107	105		
12	7	20	98	114	85	94	98	96	
12	7	21	58	102	73	103	84		111
12	8	22	66	90	94	97	87		
12	8	23	112	195	137	161	151	125	
12	8	24	126	207	86	131	137		
12	13*	25	140	174	149	132	149		
12	13*	26	112	143	111	174	135	132	132
12	13*	27	109	115	110	117	113		
18	9	28	37	126	93	139	99		
18	9	29	86	59	91	62	74	84	
18	9	30	70	94	75	72	78		95
18	10	31	91	82	84	77	84		
18	10	32	87	95	90	111	96	105	
18	10	33	134	166	116	129	135		
25	11	34	42	40	49	31	41		
25	11	35	41	30	39	53	41	42	
25	11	36	34	34	60	51	45		35
25	12	37	40	29	41	16	32		
25	12	38	22	37	29	25	29	28	
25	12	39	20	24	35	20	25		

* Died of dissecting aneurysm

APPENDIX II - B

PRECIPITATED PHOTOGRAPHIC GRAINS PER OIL-IMMERSION-FIELD IN AORTIC MEDIAS OF NORMAL CONTROL ANIMALS. COMPARE TO FINDINGS OF LATHYRITIC ANIMALS AS LISTED IN APPENDIX II - A.

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
1	17	40	108	166	164	120	139		
1	17	41	117	110	131	108	116	102	
1	17	42	89	93	116	111	102		107
1	18	43	64	85	91	79	80		
1	18	44	88	68	96	72	79	95	
1	18	45	137	121	124	126	127		
3	19	46	52	98	56	71	69		
3	19	47	147	59	66	37	77	88	
3	19	48	154	109	101	99	116		78
3	20	49	36	49	41	54	45		
3	20	50	75	73	97	86	83	68	
3	20	51	64	67	76	100	77		
7	21	52	32	37	22	25	29		
7	21	53	31	43	48	60	46	44	
7	21	54	90	46	60	37	58		39
7	22	55	29	20	18	25	23		
7	22	56	36	24	51	37	37	33	
7	22	57	52	49	59	40	50		

APPENDIX II-B -- (CONTINUED)

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
12	23	58	33	41	35	31	35		
12	23	59	40	35	43	23	36	32	
12	23	60	36	18	27	16	24		30
12	24	61	6	10	19	9	11		
12	24	62	46	51	40	46	46	29	
12	24	63	45	30	22	26	31		
18	25	64	13	12	16	9	13		
18	25	65	17	10	10	7	11	11	
18	25	66	5	10	11	8	9		8
18	26	67	4	3	4	1	3		
18	26	68	0	10	3	6	5	5	
18	26	69	6	8	4	7	6		
25	27	70	24	14	33	17	22		
25	27	71	15	21	11	10	14	16	
25	27	72	17	10	9	5	10		11
25	28	73	7	5	11	6	7		
25	28	74	4	5	2	5	4	6	
25	28	75	12	5	6	5	7		

APPENDIX III-A

PRECIPITATED PHOTOGRAPHIC GRAINS PER OIL-IMMERSION-FIELD IN XIPHOID PROCESS OF LATHYRITIC ANIMALS. COMPARE TO FINDINGS OF NORMAL CONTROL ANIMALS AS LISTED IN APPENDIX III-B.

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
1	1	1	376	339	373	370	364		
1	1	2	708	520	713	510	613	500	
1	1	3	470	519	560	536	522		612
1	2	4	575	621	675	736	652		
1	2	5	937	702	624	587	713	723	
1	2	6	1144	666	668	733	803		
3	3	7	456	422	542	411	458		
3	3	8	296	288	212	270	267	343	
3	3	9	292	292	284	342	303		417
3	4	10	702	643	533	637	629		
3	4	11	495	316	338	338	372	491	
3	4	12	456	441	414	574	471		
7	5	13	484	380	502	485	464		
7	5	14	458	428	484	440	452	463	
7	5	15	414	516	449	520	474		415
7	6	16	385	436	471	339	408		
7	6	17	258	290	291	230	217	366	
7	6	18	306	352	398	341	474		

APPENDIX III-A -- (CONTINUED)

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
12	7	19	190	213	240	233	219		
12	7	20	244	232	216	293	221	242	
12	7	21	215	349	316	263	286		306
12	8	22	107	215	122	198	160		
12	8	23	365	446	393	393	399	369	
12	8	24	614	716	384	495	550		
12	13*	25	196	375	265	389	306		
12	13*	26	238	235	212	260	231	265	265
12	13*	27	277	226	256	275	259		
18	9	28	432	386	382	371	393		
18	9	29	425	311	428	351	379	360	
18	9	30	377	278	275	302	308		340
18	10	31	317	372	348	358	349		
18	10	32	348	253	228	218	261	319	
18	10	33	275	354	367	387	348		
25	11	34	266	270	176	207	229		
25	11	35	137	183	171	167	164	188	
25	11	36	144	143	207	200	173		110
25	12	37	40	58	30	27	39		
25	12	38	33	15	20	10	19	31	
25	12	39	36	32	38	37	36		

*Died of dissecting aneurysm

APPENDIX III-B

PRECIPITATED PHOTOGRAPHIC GRAINS PER OIL-IMMERSION-FIELD IN XIPHOID PROCESS OF NORMAL CONTROL ANIMALS. COMPARE TO FINDINGS OF LATHYRITIC ANIMALS AS LISTED IN APPENDIX III-A.

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
1	17	40	512	378	561	391	461		
1	17	41	310	379	245	311	316	388	
1	17	42	426	366	398	342	383		366
1	18	43	385	441	362	356	388		
1	18	44	368	391	377	468	401	395	
1	18	45	645	742	662	793	383		
3	19	46	338	484	406	470	424		
3	19	47	421	475	341	426	416	492	
3	19	48	604	706	589	651	637		385
3	20	49	145	168	125	212	162		
3	20	50	348	467	381	362	399	278	
3	20	51	290	263	242	277	268		
7	21	52	-*	-	-	-	-		
7	21	53	-*	-	-	-	-		
7	21	54	-*	-	-	-	-		531
7	22	55	562	527	423	427	485		
7	22	56	421	466	564	547	499	531	
7	22	57	539	766	545	583	608		

* Tissue not included on slide

APPENDIX III-B -- (CONTINUED)

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
12	23	58	389	379	414	368	387		
12	23	59	360	327	399	461	387	379	
12	23	60	309	353	399	399	365		351
12	24	61	285	305	342	288	305		
12	24	62	299	325	353	451	356	322	
12	24	63	268	329	238	389	306		
18	25	64	118	126	159	128	133		
18	25	65	135	183	160	182	165	149	
18	25	66	153	131	145	172	150		139
18	26	67	119	101	85	106	103		
18	26	68	122	97	158	132	127	128	
18	26	69	197	127	123	184	155		
25	27	70	108	144	132	169	138		
25	27	71	113	120	119	89	110	98	
25	27	72	68	29	48	36	452		81
25	28	73	82	28	65	31	515		
25	28	74	59	28	26	36	37	64	
25	28	75	112	88	113	99	103		

APPENDIX IV - A

PRECIPITATED PHOTOGRAPHIC GRAINS PER OIL-IMMERSION-FIELD IN MUSCULAR DIAPHRAGM OF LATHYRITIC ANIMALS. COMPARE TO FINDINGS OF NORMAL CONTROL ANIMALS AS LISTED IN APPENDIX IV-B.

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
1	1	1	11	27	22	22	21		
1	1	2	35	39	48	44	42	32	
1	1	3	42	24	32	40	35		34
1	2	4	12	36	15	9	18		
1	2	5	58	62	29	32	45	36	
1	2	6	36	37	62	46	45		
3	3	7	17	22	26	20	21		
3	3	8	9	2	10	10	8	13	
3	3	9	11	10	14	9	11		10
3	4	10	4	13	6	5	7		
3	4	11	8	7	7	5	7	8	
3	4	12	9	12	8	9	9		
7	5	13	0	8	15	4	7		
7	5	14	9	5	16	13	11	9	
7	5	15	11	7	7	7	8		8
7	6	16	8	9	7	4	7		
7	6	17	15	12	13	6	12	7	
7	6	18	7	11	15	12	11		

APPENDIX IV-A -- (CONTINUED)

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
12	7	19	8	7	6	7	7		
12	7	20	6	3	10	9	7	7	
12	7	21	7	7	5	6	6		
12	8	22	2	2	2	2	2		5
12	8	23	2	4	8	5	5	4	
12	8	24	7	3	3	6	5		
12	13*	25	10	6	9	5	8		
12	13*	26	5	9	5	9	7	9	9
12	13*	27	10	10	10	14	11		
18	9	28	2	6	8	5	5		
18	9	29	4	8	3	3	5	5	
18	9	30	4	3	4	3	4		
18	10	31	3	2	5	4	4		4
18	10	32	2	3	1	2	2	4	
18	10	33	1	8	8	9	7		
25	11	34	2	2	0	4	2		
25	11	35	5	5	4	1	4	3	
25	11	36	4	4	6	2	4		
25	12	37	2	2	3	2	2		4
25	12	38	2	2	3	2	2	4	
25	12	39	5	5	4	1	4		

*Died of dissecting aneurysm

APPENDIX IV - B

PRECIPITATED PHOTOGRAPHIC GRAINS PER OIL-IMMERSION-FIELD IN MUSCULAR DIAPHRAGM OF NORMAL CONTROL ANIMALS. COMPARE TO FINDINGS OF LATHYRITIC ANIMALS AS LISTED IN APPENDIX IV-A.

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
1	17	40	41	25	46	52	41		
1	17	41	11	6	19	22	15	27	
1	17	42	21	23	22	23	25		19
1	18	43	8	2	10	6	7		
1	18	44	8	13	7	13	10	11	
1	18	45	25	31	31	19	17		
3	19	46	8	5	2	5	5		
3	19	47	3	9	5	6	6	5	
3	19	48	5	5	8	4	6		5
3	20	49	4	3	7	9	6		
3	20	50	1	1	5	1	2	5	
3	20	51	7	7	4	5	6		
7	21	52	3	2	4	0	3		
7	21	53	8	12	4	4	7	5	
7	21	54	9	4	6	2	5		11
7	22	55	30	39	30	33	33		
7	22	56	12	9	1	6	7	16	
7	22	57	6	15	8	5	9		

APPENDIX IV-B -- (CONTINUED)

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
12	23	58	6	3	6	8	6		
12	23	59	5	4	4	12	6	7	
12	23	60	10	8	10	12	10		
12	24	61	3	1	1	2	2		5
12	24	62	3	2	2	0	2	2	
12	24	63	5	2	4	3	4		
18	25	64	5	1	5	4	4		
18	25	65	1	4	2	0	2	3	
18	25	66	3	6	6	2	4		
18	26	67	0	5	0	2	2		3
18	26	68	1	0	1	2	1	2	
18	26	69	3	4	2	2	3		
25	27	70	3	6	1	5	4		
25	27	71	2	4	3	4	3	3	
25	27	72	3	2	0	1	2		
25	28	73	6	10	4	1	5		4
25	28	74	3	1	13	3	5	5	
25	28	75	3	5	2	4	4		

APPENDIX V

PRECIPITATED PHOTOGRAPHIC GRAINS PER OIL-IMMERSION-FIELD IN AORTA, MUSCULAR DIAPHRAGM, AND XIPHOID PROCESS OF CONTROL ANIMALS WHICH RECEIVED NO S^{35}. BACKGROUND CONTROLS.

(1) AORTIC MEDIA

Day Post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
-	29	76	7	6	9	4	7		
-	29	77	8	3	7	4	6	7	
-	29	78	6	6	8	10	8		5
-	30	79	4	5	3	5	4		
-	30	80	2	2	1	2	2	4	
-	30	81	5	5	4	6	5		

(2) XIPHOID PROCESS

Day post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
-	29	76	4	5	4	3	4		
-	29	77	11	9	10	7	9	7	
-	29	78	4	8	8	9	7		7
-	30	79	7	6	4	6	6		
-	30	80	7	5	6	3	5	7	
-	30	81	12	5	12	8	9		

APPENDIX V -- (CONTINUED)

(3) MUSCULAR DIAPHRAGM

Day Post S^{35} inj.	Animal No.	Slide No.	Grains per oil-immersion-field				Average grains per slide	Average grains per animal	Average grains per sacrifice day
-	29	76	5	4	2	4	4		
-	29	77	5	3	2	4	4	4	
-	29	78	5	5	8	4	6		
-	30	79	3	4	1	1	2		4
-	30	80	2	2	2	2	2	3	
-	30	81	3	4	5	2	4		

APPENDIX VI

BRIEF HISTORY OF HUMAN DISSECTING ANEURYSM

Traditionally, the term "dissecting aneurysm" is taken to imply a separation of the elements of the media, or a separation of the media from the adventitia with or without an accumulation of blood. Almost all such dissections begin in the thoracic aorta, are characterized by excruciating and tearing pain, aortic diastolic murmurs, secondary neurological disorders in the lower extremities, and result in death due to massive hemorrhage within 48 hours (71). The method by which dissection occurs has been a source of controversy in the past. If we exclude the traumatic, neoplastic, infectious, and foreign body erossive conditions, there are two different schools of thought that have been advocated over the years.

The older concept, and the one that is presently falling into disfavor, was that the process leading to dissecting aneurysm began with atheromatous degeneration and was intimately associated with increased intravascular pressure. Lipids were known to be deposited in the intima, and as a result there was a heaping up of the overlying intima. There followed deposition of calcium salts, and the aortic wall was converted into a stiff rigid tube, which was fragile and indeed cracked in areas of the plaques. This was said to result in blood

being forced, by the abnormally high aortic pressure, into these defects. With time and continued atheromatous degeneration, the blood penetrated the media. In the cases where dissection occurred in young people without evidence of arteriosclerosis, the hypertension alone was considered enough to cause the disease.

In 1932 Moritz (4) attempted to produce a dissection of the aorta by injecting a solution into that vessel wall in young living rabbits. He reached pressures of 800 to 1200 mm of mercury without causing a rupture. Koltz and Simpson (5) in 1932 subjected human aortas from autopsy specimens between the ages of twenty and forty to pressures of 1000 mm of mercury without causing any damage. From these and other experiments, it was realized by many investigators that the long present hypertension noted in about half of the people who died of dissecting aneurysms did not reach a fraction of the pressure necessary to cause a rupture of the aorta. Even in patients with severe atheromatous plaques, although it has been possible in some experiments on autopsy specimens to cause an aortic rupture at a severely calcified and degenerative area (3), the pressures required to do so are extremely high, and are never achieved in life. Oppenheim in 1918 was able to demonstrate one such rupture in a severely degenerated aorta from a sixty-two-year-old woman. The rupture occurred at the site of a calcified plaque, but the pressure necessary to do so exceeded 790 mm of mercury (3).

Erdheim (19) in 1929 and 1930 noted the appearance of mucoid "cystic pools" in the media of aortas in individuals having severe degeneration of this vessel. These cystic accumulations of mucoid-like material had been observed by many other workers in previous years, but until Erdheim's work they had been considered to be secondary to necrosis of the tissues of the media. Erdheim concluded that these cystic areas, which appeared like small lakes in the media, were not the result of necrosis, but indeed were the very origin of the degeneration of the aortic media.

Orsos (72) in 1931 made a detailed study of the aortic media and demonstrated the elastic laminae embedded in collagen and surrounded by numerous precollagenous fibrils. The interlaminar spaces contained muscle cells and a fine mucoid material which he proposed acted as the "embedding lubricant" for the various other elements. The media alone was considered structurally endowed to give the strength and resistance against circulatory pressures. Because of Erdheim's observations of accumulations of mucoid-like material, and assisted with the scrupulous study of the normal aorta as performed by Orsos, attention was turned to the media as being the primary site for initiation of the dissecting process. Thus began the second school of thought, and the one that is generally accepted today, that the primary process in dissecting aneurysm is a degeneration and a resulting weakening of the structures in the media.

Due to the awakening interest in the 1930's of the specific site and etiology of aortic media degeneration, attention was turned back to some of the important but overlooked observations made by Wiesel (20) in 1906 and 1907 on the media of various sized arteries of young patients observed after death from acute fulminating infections. This author described the accumulation of a homogenous acellular substance between the elastic laminae of such patients. The infectious diseases studied by Wiesel included pneumonia, influenza, typhoid, and diphtheria. In addition to the mucoid-like accumulations, he described signs of degeneration of the elastic fibers, and of the smooth musculature. There was no reference made to dissecting aneurysms.

In the 1930's and early 1940's many investigators began to report findings of numerous cyst-like spaces filled with a mucoid-type material located in the aortic media of patients dying from dissecting aneurysm. Some of these include: Woff (13), Narr and Wells (14), Roberts (15), Glendy, Castleman and White (16), Shennan (17), and Holland (18). The majority of these investigators described this finding, along with elastic tissue and muscle degeneration, as being most intense in the ascending and aortic arch.

In 1942 Sailer (3) described what were considered to be five types of medial lesions: 1) primary degeneration of the elastic lamella with fragmentation and associated damage to the supporting collagen, with or without accumulation of

"mucoid" material in cystic areas; 2) hyaline degeneration of the connective tissue elements between the main elastic lamina; 3) atrophy of muscle with fatty degeneration; 4) muscle necrosis with extensive structureless homogenization of the muscle cells with and without "mucoid cysts;" 5) primary overproduction of a mucoid-like material in the interlamellar ground substance with "cyst" formation and secondary disruption of muscle, elastic tissue, and collagen fibers. He made the further observations that the mucoid substance so frequently seen appeared to be the result of a developmental disturbance, or a result of aging, or perhaps a reaction to some unknown toxic stimulus. No single cause could be incriminated as being the agent leading to vascular change and resultant dissecting aneurysm.

In 1952 and 1953 Gore reported that the greatest incidence of dissecting aneurysms of the aorta was in the forty to sixty age group, with males being twice as susceptible as females. Of seventy-two cases studied by him, thirty-two occurred in patients under forty years of age, and medial degeneration was common to all of them. He considered hemorrhage from the vasa vasorum to be important in the initiation of the lesion (6, 7).

Gore's observation that medial degeneration and dissecting aneurysm occurred in young people who frequently did not have hypertension nor any evidence of arteriosclerosis had been observed before in several case reports, including

a twelve-day-old infant (73), a fourteen-month-old male (74), and a ten-year-old boy (74).

An interesting case report of a dissecting aneurysm that occurred ten days post partum in a woman with no history of hypertension was that of Schnitkee and Bayer (2). They demonstrated on microscopic section that the media had a decreased amount of elastic tissue and that there were frequent small cystic areas of accumulation of a "mucin-like material." After reviewing the literature up to 1944, these authors discovered that there were 141 reported cases of dissecting aneurysm that occurred in patients under the age of forty, and these represented about twenty-five percent of all reported cases. About one-half of these one-hundred-forty-one cases had hypertension. They concluded that hypertension was not the etiologic agent, but that the dissection was secondary to cystic medial necrosis of unknown cause which resulted in a softening and weakening of the media leading to rupture of the small nutrient vessels and hematoma formation. The resulting enlargement of the vessel wall was said to lead to a stretching of the overlying intima with secondary pressure necrosis causing the intima to tear from without inward. This opening then allowed the aortic blood to enter under pressure and to further dissect the weakened media.

More recently Hume and Krosnick (9) reported the successful treatment of a thirty-two-year-old pregnant woman who developed a dissecting aneurysm at full term with resulting

left iliac artery occulsion and acute aortic insufficiency. The distal tear was repaired immediately and the fetus delivered by cesarean section. The proximal tear was repaired three weeks later with good results. These authors suggested that early diagnosis of a pre-dissecting state may be possible if one was constantly aware that the association of either hypertension, Marfan's Syndrome, coarctation of the aorta, or a generalized "collagen disease" along with pregnancy should make one have a higher index of suspicion and to hence investigate more thoroughly, not only physically, but by delving into the family history for possible deaths due to dissecting aneurysms.

The association of medionecrosis of the aorta, leading to dissecting aneurysms, and Marfan's Syndrome, has recently been reviewed by Tung and Liebow (10). These authors pointed out the marked loss of elastic tissue and the presence of small mucoid, acellular cysts in the aortic media. Atta and Hock (11) reported the same observations. Many of the traits of Marfan's Syndrome suggest that the basic defect is an alteration of the connective tissues of the body, and indeed studies upon the serum levels of muco-polysaccharides in such patients suggest that the metabolism of the ground substance is abnormal. Bacchus (12) reported a significant decrease in the serum mocopolysaccharides of six such patients. This is in contrast to the findings of other investigators as summarized by Rich (75) that the serum and

urine levels of acid mucopolysaccharides are increased in rheumatoid arthritis, lupus erythematosus, cancer, leukemia, and various other malignant diseases in which there is considered to be abnormal connective tissue metabolism.

Hukill in 1957 (8) reported an interesting finding of a "healed dissecting aneurysm" associated with extensive evidence of cystic medial necrosis in a forty-three-year-old male who died of unrelated causes, and who was not hypertensive nor had significant arteriosclerosis.

It should be pointed out that not all investigators consider the accumulation of mucopolysaccharides in the aortic media as abnormal, although there are conflicting ideas on this matter. Bunting has studied extensively the ground substance of the aorta (76, 77), and when he analyzed it with respect to age, he reported that there seemed to be a "normal"increase of the mucopolysaccharides with increasing age until the fourth decade, and that there were even "mucous cysts" in some "normal" aortas of older patients (78). This is in direct contrast to the more recent findings of several other investigators (79, 80, 81) all of whom found there to be no correlation with increasing age. In spite of the lack of agreement concerning the state of medial mucopolysaccharides in the "normal" aging patient, there seems little doubt but what the metabolism of the ground substance is altered in conditions of dissecting aneurysm of unknown cause (13, 14, 15, 16, 17, 18).

Burman (1) in 1960 summarized the evidence which had accumulated against the old classical concept of atheromatous degeneration as being the pathogenesis of all non-traumatic dissecting aneurysms. This evidence was as follows: 1) dissecting aneurysms often occur in young people who do not have hypertension, arteriosclerosis, or intimal disease; 2) the pressure required to burst the aorta which has a normal, or even abnormal, media is impossible to achieve in life; 3) it has been impossible experimentally to cause a dissecting aneurysm to occur by injecting blood into the intima or media; 4) dissection of the media occurs even when there is no opening in the intima for blood to enter; 5) separation of the elements of the aortic media occur beyond the limits of the "dissecting column of blood" in many cases; 6) a complete dissection may occur in which there is absolutely no blood found, and yet there is extensive splitting of the elements of the media. Hence there would appear to be rather profound metabolic and structural changes occurring in the connective tissues of the aortic media of such patients.

APPENDIX VII

REVIEW OF THE LITERATURE

Human lathyrism

Lathyrism in human beings appears to be essentially a disease of the central nervous system. As early as the time of Hippocrates, neurolathyrism had been accurately described (23). He observed spastic paralysis of the legs after the inclusion of sweet peas in the diet following a famine. It has been known for centuries in India that this disease was caused by eating various types of seeds from species related to the flowering sweet pea (30), and rather sporatic epidemics have been reported in the medical literature (82, 83).

A recent epidemic of human neurolathyrism occurred in a rural area near Calcutta, India, in 1960. The people in this section of the country had been eating lathyrus seeds for five to six months of the year over a period of six years because of heavy spring floods which made it impossible to raise rice (29). The symptoms noted were: prodromal painful spasms, cramps of the calf muscles, and urinary incontinence for several days to several months before the onset of paralysis of the lower limbs. Other symptoms frequently present, along with the leg paralysis, were fecal incontinence and sexual impotence. Signs included exaggerated deep tendon

reflexes in lower extremities, spastic gait, absent cremasteric and abdominal reflexes. Improvement was observed on return to normal diet. Males were mostly affected.

The variety of the sweet pea that has caused most of the epidemics in India has been identified as Lathyrus sativus. In other countries other species have been identified including Lathyrus cicera in France, and Lathyrus clymenum in North Africa (58).

It is not known whether the toxin that has been shown to be the cause of lathyrism in rats fed on Lathyrus seeds is the same one causing the neurological manifestations in humans. Indeed, some investigators attribute the human disease to an antigen-antibody reaction (29). In 1961 Ressler, Redstone, and Erenberg (84) reported the first isolation and identification of a neurotoxic factor for the rat from Lathyrus latifolius and Lathyrus sylvestris Wagneri. This was the substance L-alpha, gamma-diaminobutyric acid. No attempt has been made, as of yet, to identify a similar or related substance from those species known to cause human lathyrism.

The lathyritic agent

In 1934, Stockman, who had done some of the earliest experimental work on lathyrism (85), undertook to study the chemistry and pharmacology of the sweet pea in an attempt to discover what the specific agent was that was causing the

bony lesions (86). He was able to extract the lathyritic agent in a crude form from the sweet pea seed with water, and referred to it as a "phytic acid."

Not much more headway was made in identifying the agent more exactly until in 1954 when Dupey and Lee (87) reported the isolation from the Lathyrus pusillus seeds of a crystalline substance that produced the skeletal deformities other investigators had noted by feeding whole seeds (21, 41). Later that same year Schilling and Strong (88) isolated and identified from Lathyrus odoratus a crystalline substance beta-(gamma-L-glutamyl)-aminopropionitrile that produced identical lesions, in rats and other animals, to those previously described.

In 1961 Levene (89) did extensive studies on the varieties of nitriles and related substances that could produce lathyrism and reported that there were four groups of compounds having the potential to induce the disease. He used chick embryos, and measured the ability of these substances to produce solubilization of collagen, and also skeletal deformities. These four groups of compounds in order of diminishing potency were: 1) nitriles, 2) ureides, 3) hydrazides, and 4) hydrazines.

Gardner (90) points to the striking similarity in chemical structure of several of the lathyrus producing nitriles and nitrogen containing compounds. Several of the chemical structures of these substances follow:

$COOH\text{-}CNH\text{-}CH_2\text{-}CH_2\text{-}CO\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}CN$

beta-(N-gamma-(glutamyl)-aminopropionitrile

$NH_2\text{-}CH_2\text{-}CH_2\text{-}CN$

beta-aminopropionitrile

$NH_2\text{-}CH_2\text{-}CH$

aminoacetonitrile

$CH_2\text{-}N\text{-}CH_2\text{-}CN$

methyleneminoacetonitrile

$NH_2\text{-}CH_2\text{-}CH_2\text{-}S\text{-}H$

beta-mercaptoethylamine (cysteamine)

$NH(CH_2\text{-}CH_2\text{-}CN)_2$

bis-(beta-cyanoethyl)-amine

$NH_2\text{-}NH\text{-}CO\text{-}NH_2$

semicarbazide

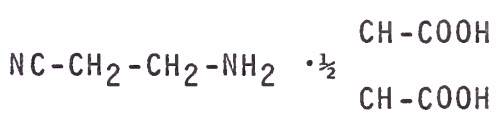
$(CH_3)_2\text{-}CN\text{-}NH\text{-}COH_2$

acetone semicarbazone

The neurotoxic factor isolated from Lathyrus latifolius and Lathyrus sylvestris Wagneri by Ressler, et al., in 1961 (84)

bears a striking similarity to the synthetically prepared beta-aminopropionitrile that is believed to be the lathyritic agent in the widely used Lathyrus odoratus seed in experimental lathyrism. Indeed, Ressler, et al., propose a common pathway for the two substances for aspargine to an intermediate beta-cyano-L-alanine which in turn is decarboxylated to beta-aminopropionitrile in Lathyrus odoratus, or reduced to L-alpha, gamma-diaminobutyric acid in Lathyrus latiforius and Lathyrus sylvestris Wagneri.

Beta-aminopropionitrile fumarate, the compound used to produce lathyrism in this study, is a pure white crystalline salt which is soluble in water, alcohol, and alkali. The melting point is 172° - 173° C. The molecular weight is 128.2 (91).



beta-aminopropionitrile fumarate

The effects of numerous agents on the rate of development and the severity of lathyrism have recently been summarized by Selye (82), and he notes that the following appear to increase the severity of the disease: hepatectomy, growth hormone; and the following substances decrease the severity: thyroxine, ACTH, thyroid stimulating hormone, and estrogens.

Experimental Lathyrism

Experimental lathyrism appears to be a generalized disorder of the connective tissue of the body, in addition to rather characteristic neurological effects, and has attracted a great deal of attention over the years. One of the earliest planned scientific experiments was that of Stockman (85) in 1917 who studied lathyrism as induced in rabbits and monkeys by feeding seeds from the flowering sweet pea plant. His observations centered around the nervous system symptoms, and he reported spasmodic attacks with hind leg paralysis in monkeys and death due to respiratory muscle failure. He noted the same spasmodic symptoms in the legs of guinea pigs and rabbits associated with weakness and finally death. Stockman attributed the condition to a toxic factor, an alkaloid, in the lathyrus peas. There is no mention in Stockman's work of aortic aneurysms.

Several other investigators reported similar findings to those of Stockman's, including Howard (24) in 1923 and McCarrison (25) in 1927. In 1933 Geiger (92) completed extensive studies on experimental lathyrism in the rat and reported the first observations of gross skeletal deformities. He demonstrated scoliosis of the spine in the thoracic region, sternal deformities, malformation of the femur and hypertrophy at the costochondral junction. He was able to extract the toxic agent from the lathyrus peas by the use of

boiling water.

Recently attention has again been turned to experimental lathyrism by Ponseti and Baird in 1952 (41), Ponseti and Shepherd in 1954 (21), Ponseti and Bean in 1955 (43), and Bachuber and Lolich in 1955 (42), and has resulted in numerous investigators becoming interested in this enity (44, 45, 46, 47, 89, 90, 88). In the classic experiment of Ponseti and Baird in 1952, (41) four-week-old rats were started on a diet consisting of 50% ground Lathyrus odoratus seed. After being on this diet for 2-3 weeks the authors noted demineralization and deformities of the long bones of the body with secondary new bone formation at the level of femoral metaphysis. At 4-5 weeks there was slipping and irregularities of the intervertebral spaces. At 6 weeks severe kyphoscoliosis began to appear in the thoracolumbar region. At 7 weeks there were severe costal deformities. In addition the animals were noted to be thin and listless with rumpled fur. Paraplegia developed in a few of the animals who developed dragging of the hind quarters. If the animals were replaced on the normal diet, they were noted to become fat, sleek, and healthy-appearing again. One-third of the rats died of massive hemorrhage from dissecting aneurysms of the aorta between the 5th to the 9th weeks. Sections of these aortas revealed widespread medial necrosis along the thoracic aorta and dissection and disruption of the fibers in the media.

Ponseti and Shepherd in 1954 (21) produced lathyrism in rats by use of Lathyrus odoratous seeds and noted the following lesions: widening of epiphyseal plates with grouping of cartilage cells and loss of cohesion of the cartilage matrix. There was also loosening of the tendinous and ligamentous insertions. Further observations revealed epiphyseal slippings, metaphyseal fibrosis, kyphoscoliosis, subluxations, dislocation of the shoulder joint, degeneration of intervertebral disc, disc herniation, Legg-Perthes-like disease of femoral head, valgus of forepaws, massive dissecting aneurysms of the aorta and inguinal hernias. They concluded that the defect in the ground substance was due to excessive destruction of the chondroitin sulphate as manifest by the lesions both in the aorta and the skeletal system.

Bachhuber and Lolich in 1955 (42) induced lathyrism in rats by feeding a water-alcohol extract of the sweet pea meal and noted similar skeletal and bone findings to those of Ponseti. He reported that over 50% of his animals died of ruptured aortic aneurysms that were confined to the ascending and transverse arch. He also noted edema of the media and fragmentation of the elastic fibers with intimal fibrosis. The dissecting aneurysms occurred during the 27 - 68 days of the diet.

Churchill and others in 1955 (44) studied the aortas of lathyritic rats fed with 50% Lathyrus odoratus and reported extensive hemorrhages in the media and dissecting

aneurysms. There was an increased amount of metachromasia and periodic acid-Schiff positive material between the elastic fibers in the media as had been reported by others. The investigators proposed that the polysaccharide they were demonstrating in increased amounts was chondroitin sulphate B. In addition they noted degeneration of elastic fibers with fragmentation in the media of the aortic wall. Their hypothesis was that the site of action of lathyritic agents was on the mucopolysaccharide of the ground substance resulting in altered structure and leading to weakness of the aorta. Their proposal was in agreement with that of Bean and Ponseti (43) that the aortic lesions were due to excessive destruction of chondroitin sulphate.

The true nature of the lathyritic defect was admittedly not understood by these initial investigators (21, 41, 42, 43, 44), although they implied that the metabolism of the mucopolysaccharides was probably at fault. However, as further investigation was undertaken, the evidence began to accumulate that at least part of the defect was indeed in the ground substance. Schwartz and Gilmore in 1958 (59) demonstrated elevation of serum mucoprotein and hexosomine levels in lathyritic animals and interpreted their findings as being consistent with depolymerization of the ground substance mucopolysaccharide in vivo.

Pyorala, Punser, and Seppola in 1958 (45) also implied that metabolism of the mucopolysaccharides was basically at

fault in lathyrism. They described an increase in the metachromosia and material stainable with alcian blue, colloidal iron and aldehyde fuchsin in the aortas of rats made lathyritic with a 50% sweet pea diet. They also demonstrated an increase in the P.A.S. positive material between the elastic fibers with disruption of the elastic laminae. In certain areas of the aorta the elastic laminae were markedly disrupted by large accumulations of metachromatic material. This material was often coarsely granulated and frequently obscured the elastic fibers. Most of these changes took place in the inner part of the media. They also described an increase in mast cells in the adventitia. This is of interest in that although the fibroblast is considered to be the side of origin of the mucopolysaccharides of connective tissue, the mast cell may possibly be involved also (77).

Most investigators consider there to be five different kinds of mucosaccharides in the ground substance of connective tissue. These are: hyaluronic acid, which is widespread throughout the body; chondroitin sulphate A, found in cartilage; chondroitin sulphate B found in the aorta, tendons, and skin; chondroitin sulphate C which is widespread in most connective tissues; and hyaluronosulphate, which is found only in the cornea (93). It is felt by most investigators that the increased metachromatic material found in the aortas of lathyritic animals is the chronroitin sulphate B variety (44).

In 1956 Walker and Wirtschafter (46) did extensive histological studies of the aortic lesions in lathyrism induced in rats by the 50% sweet pea meal, and found that the entire aorta from its origin into the abdomen was involved with many microaneurysms, medial hemotomas, and massive displacement of the elastic fibers by fibroblastic proliferation. Indeed there had been a few animals die of ruptured aneurysms of the abdominal aorta. In addition, they found that the pulmonary and coronary arteries were involved showing increased thickness, decreased number and lysis of elastic fibers, and fibroblastic proliferation to the same degree as did the aorta. However, they found aneurysms only in the aorta. The proliferation of fibroblasts was considered to be a reparative process.

Wirtschafter repeated his initial experiments in 1957 (47) and demonstrated hyperplasia and hypertrophy of the fibroblasts, many containing atypical nuclei. He observed that the increased staining for acid mucopolysaccharides (Hale's stain) occurred at the same sites where there was an increase in the number and size of fibroblasts in the aorta, pulmonary and coronary arteries. The fibroblasts are considered the source of mucopolysaccharides (76). He also noted that there was a greater increase in the aortic media intercellular P.A.S. positive staining material near the site of aneurysms than where there was no rupture. This P.A.S. positive material appeared, too, in highest concentration

along the surface of the elastic laminae.

The important finding by Walker and Wirtschafter (46), Bean and Ponseti (43), Pyorola, et al. (45) that there was an increase in the acid mucopolysaccharide in the aortas of lathyritic rats was further confirmed by Menzies and Mills in 1957 (55).

Schwartz in 1959 (38) studied the aortas of rabbits made lathyritic with daily subcutaneously injections of beta-aminopropionitrile and demonstrated an accumulation of metachromatic material, and also some P.A.S. positive substance, between widely separated and disrupted elastic fibers of the media. He concluded that the increased metachromosia represented abnormal depolymerization of the ground substance. This increase in the basic units of the mucopolysacchrides was said to result in a secondary increase in the serum mucoprotein and hexosamine levels that had been noted by other investigators (59). It is also of interest that this author (38) was able to show an increased number of atherosclerotic lesions of a more severe nature in those rabbits that received cholesterol in their diets as opposed to non-lathyritic animals which also received cholesterol diet. Previous investigators have shown (94, 95, 96) that there may be an increase in the metachromatic material in human atheroscleratic degeneration, although other investigators have found no such relationship (79, 80, 81), and Schwartz puts forth the hypothesis that such individuals

may have ground substance depolymerization just as did the lathyritic rabbits.

In 1960, Grant, Hathorn, and Gillman (97) demonstrated a marked rise in the aortic hexosamine and uronic acid without any change in the hydro-oxyproline of lathyritic rats. They were unable to demonstrate any change in the hexosamine and uronic acid levels in the skin and femur. They postulated that the increased mucopolysaccharide in the aorta was not due to destruction of components of elastic fibers but to increased synthesis and discharge of mucoprotein. They suggested that the greater accumulation of mucopolysaccharides, noted around the surface of elastic fibers by themselves and others (46), might imply a block in the neogenesis of elastin and collagen-like fibrillar components of the elastic tissue. These findings are in agreement with Walker (60), Bauer and Carlsson (39), and Karnovsky (48), who all agree that one of the basic defects in lathyrism involves the mucopolysaccharides.

Electron microscopic examination of the lathyritic rat aorta gives added evidence and is in agreement with previous observations that there is an increase in the intercellular space and ground substance (54). In addition there has been reported vacuolated muscle cells, enlargement and dilatation of the Golgi apparatus, endoplasmic reticulum and mitochondria. The edges of the elastic fibers are seen to change from being nice and sharply defined to blurred and

granular with an accumulation of elastic fiber fragments.

The radiographic change in experimental lathyrism have recently been summarized by Kundel (49). The first roentographic detectable change appears to come shortly after the first observed microscopic change and occurs at about two weeks. It consists of a widening and irregularity of the epiphyseal plate. This is in agreement with microscopic and histological studies of the epiphysis (69). In addition, microscopic examination at this time shows marked cellular disorganization (31, 21, 45). The epiphyseal changes are followed by new bone formation at the periosteum involving the long bones of the body where muscles and ligaments attach. Ponseti noted this new bone formation and believed it was secondary to the slipping of the ligamentous insertion (21, 41). One week after the epiphyseal widening, slipping of the plate was noted. Concurrent with this stage, Yurika (69) reports seeing microscopic tears and clefts in the cartilage matrix. It is interesting to note here that Ham (98) has shown in rachitic rats the laying down of calcium in the cartilage matrix near the epiphyseal plate is inhibited as early as 24 hours after beginning aminoacetonitrile injections.

It is also of interest to note that the lesions in the epiphyseal plate cartilage have been shown to be histologically completely reversible if the lathyritic agent is taken away (99).

Osteoporosis begins to develop, as does kyphoscoliosis, in most of the experimental animals during the third to fourth week. Cortical atrophy occurs with occasional loss of bony architecture and replacement by spongy bone (49). The severe kyphosis may cause spinal cord compression resulting in hind leg paralysis that is similar to the direct toxic effect of the lathyritic factor upon the spinal cord. There is also wedging of the vertebrae caused by osteoporosis.

The findings of Kundel (49) are in general agreement with the lathyritic changes in the skeletal system observed by other investigators (21, 41, 43). However, Bergguist (100) did a microradiographic study on the bones and teeth in rat osteolathyrism and found no change in the teeth except for some irregularity in the pattern of dentine mineralization. There were no mineralization defects in the bones even though there were gross deformities.

Collagen defects

Not all investigators agree that the basic lesion in experimental lathyrism is an altered metabolism and structure of the acid mucopolysaccharides, even though most agree that this substance indeed, is somehow affected. The alternative view is that lathyritic agents exert their primary effect by interferring with collagen formation and that perhaps the increase in mucopolysaccharides is secondary to this.

Wirtschafter, Zolton, Bently, and Peters in 1962 (50) were able to extract abnormally high concentrations of skin

collagen from young lathyritic rats, and also found an increase above the normal in older mature experimental rats. They concluded that in lathyrism there is an interference with the normal aggregation of the newly formed collagen into insoluble fibers and this accounted for the increased concentration of extractable, soluble material. They felt that this was a more plausible explanation of their findings than the theory that there is a breakdown of pre-existing collagen fibers into smaller ones.

Martin, Gross, Piez, and Lewis, in 1961 (51) did an extensive study on the collagen in connective tissues of rats on a low protein diet made lathyritic by beta-aminopropionitrile given I. P. They injected 25 microcuries of carbon^{14} -- labeled glycine twenty-four hours before sacrifice. In the normal rats newly formed collagen is salt extractable, whereas older collagen is not and must be extracted by acid. These investigators studied the extractable collagen by chromatography, sedimentation velocity, and amino acid analysis and demonstrated a marked increase in its extractability and a pronounced loss of tensile strength. They concluded that the defect was at the molecular levels and that there was a failure to produce intromolecular cross linking between collagen fibers.

Gross (40) has shown that there is no essential difference between lathyritic collagen and normal collagen in composition of total amino acids, and carbohydrate content.

It has also been demonstrated by one that there is no difference in the X-ray diffraction of normal and lathyritic collagen (49). Gross also has demonstrated that neither denaturation temperature, intrinsic viscosity, nor flow-birefringence measurements are altered. The degree of solubility of lathyritic collagen is most striking when compared to normal, and this author also proposed that in addition to a loss of intramolecular cross linking, there may be a disruption of previously formed intermolecular bonds. Gross had previously shown (101) in vitro experiments that collagen taken from lathyritic animals does indeed form striated fibrils on warming to body temperature, but also that these same fibrils fail to become insoluble with increasing time at 37° C. incubation as does normal extracted collagen.

In contrast to the proposals of Gross, and others (102), on the failure of proper intramolecular cross linkage of collagen fibrils, is the concept proposed by Smiley, Yeazer, and Ziff (52) that newly synthesized molecules are prevented from polymerizing into collagen fibrils by the lathyritic mechanism.

Kulonew, Solmi, and Jura (56) studied the uptake of nitrogen^{15}, administered as $N^{15}H_4Cl$, in the collagen of chick embryos and demonstrated an increased uptake in the insoluble fraction of lathyritic animals over the normal controls. They concluded that the synthesis of "collagen" was actually

enhanced by aminopropionitrile administration but that the collagen in the diseased animals was more liable metabolically with increased solubility.

Normal collagen is made up of subunits called tropocollagen, or alpha collagen, secreted by fibroblasts, that polymerizes extracellularily. Alpha collagen itself is composed of three polypeptide chains coiled into a helical configuration. Further polymerization takes place outside the cell resulting in beta-collagen and finally mature collagen. The important process in this formation of mature strong collagen is the formation of bonds between the side branches of the chains (49).

Levene in 1962 proposed a mechanism of action for the lathyrogenic factors (57). He found that glyceraldehyde completely reversed (in vitro) the effect of lathrogenic agents in causing an increased extractability of collagen from the experimental animals' skin, and hence concluded that lathyritic agents produce their specific effect by blocking carbonyl groups on collagen molecules. This is said to account for the prevention of normal cross linking. He found that he could restore the polymerization by adding a competing source of carbonyl groups.

Further evidence that the lathyritic agents produce their effects by blocking the formation of cross linking is provided by the studies of incorporation of carbon ^{14} -- labeled glycine and hydroxyproline into collagen of

experimental animals (52). These studies showed high initial and persistent incorporation into the alpha collagen fraction if the carbon ^{14} is given after the animal is made lathyritic, but not if it was given before lathyrism was produced.

It is interesting that electron microscopic studies of lathyritic collagen show the collagen fibers to be normal-appearing, but with greater-than-normal variations in diameter (103, 54). In addition to this finding Ham (54) has shown that there is an increased number of collagen fibrils in the media, and they appear to be disrupted from the normal parallel arrangement. She noticed similar changes in the collagen of the adventitia.

Hausmann in 1963 (104) performed collagen extraction experiments on skins of lathyritic rats and found that when the collagen was gelled for twenty-four hours at 37° C., it redissolved to a greater extent when subsequently placed at 4° C. than did the normal collagen gels. After the lathyritic extracts were purified several times, there was no decrease in the reversibility, or the redissolving, of the lathyritic extracts; therefore, the author concludes that the defect in intermolecular bonding of lathyritic collagen was not likely to be due to an extrinsic inhibitor factor that was present in these solutions. Further evidence against such an extrinsic factor is that the author found that by adding beta-aminopropionitrile to the normal collagen extracts just prior to gelling (in vitro) there was no effect on the

reversibility in the cold. The hypothesis that the lathyritic defect is due to the absence of an extrinsic factor found in normal tissue was suggested by the author as a result of his findings that normal collagen gels become more like the lathyritic collagen solutions, in ability to redissolve on lowered temperature, after repeated purifications. However, one cannot explain the entire intermolecular defect on this basis because such purified normal collagen gels never reach the point of reversibility of the lathyritic ones, and so this author concludes that there is probably also an intrinsic defect in the lathyritic collagen molecule.

Kennedy and Kennedy in 1962 (31), after studying the uptake of S^{35}DL-methionine, C^{14} glycine, and S^{35} in the cartilage of lathyritic animals, came to the conclusion that the essential change in this disease was "a defect in the fibrogenesis due to failure of complex formation between non-collagenous protein and chondroitin sulphates A and C." This lack of combination was said to result in the weakened and structurally inferior ground substance that is constantly observed.

Sulphur-35 uptake in normal tissue

S^{35} has a half life of 87.1 days and is a weak electron emitter 0.167 Mev (105). Its use in the in vivo and in vitro study of sulphated mucopolysaccharides has been well verified (58, 69, 105, 48, 32, 53).

Dziewiatkoveski states that the maximum concentration of S^{35} in the polymucosaccharides of the normal rat tissue following I.P. injection occurs at twenty-four hours (105). When S^{35} is given in the free inorganic form to rats or mice, about 70% of it is excreted in the first twenty-four hours and almost 95% by 120 hours (106), and a fraction is retained in sulphated mucopolysaccharides (65, 66), whereas an extremely small amount is found in the amino acid cystine (65). The amount of S^{35} in cystine in rats and mice is so small that it appears these animals cannot incorporate it into amino acids by their own processes, and that which is found must be so incorporated by the action of intestinal bacteria (65). The S^{35} in amino acids, when administered in the inorganic form, is considered to be negligible in radioautographs.

The highest uptake of S^{35} is in tissues where the concentration of sulfomucopolysaccharides is known to be the highest (65, 66, 67, 68), and indeed the intensity of uptake conforms quite well to the intensity of staining with alcian blue as demonstrated by Curran (65). The S^{35} is considered to be in the esterified form as chondroitin sulphate (65, 107), and is seen in radioautographs and tissue studies to be located in the skeletal cartilage, the aorta, and the skin -- in brief, in the connective tissues of the body that contain ground substance. There is also localization of the S^{35} in: the intestine, collagen of connective tissue, spleen,

kidney, heart, and muscle. The more active tissues of young growing animals retain higher concentrations than do the older more mature tissues in older animals. The highest initial activity is found in cells that are forming mucopolysaccharides such as the gastrointestinal goblet cells, chondrocytes, fibroblasts, and mast cells (65, 108). The aortic media takes up more S^{35} than does the intima or adventitia (66).

The theory and methods of localizing radioactivity in various tissues by the use of photographic nuclear emulsion and preparing radioautographs have been well described (109, 64), as has the treatment of tissue when analyzing for bound S^{35} in mucopolysaccharides (62).

Sulphur-35 uptake in lathyritic cartilege

Most of the investigation of the connective tissue lesions of experimental lathyrism done utilizing radioactive substances has been performed on the epiphyseal plate. These studies have generally been concerned with the metabolism of the sulphated mucopolysaccharides in the ground substance of the epiphyseal plate cartilage and hence have utilized S^{35}. The results have been conflicting.

Bauer, Carlsson, and Lindquist (39) in 1955 did a study utilizing beta-aminopropionitrile induced lathyrism in rats on a significient number of animals, and sacrificed the animals eight days after injecting $Na_2S^{35}O_4$ intraperitoneally. They removed the femur, tibia, and incisor teeth and prepared

radioautographs. Their results show an increased uptake of S^{35} in the shaft of the femur of the nitrile-poisoned animals over the normal controls by two to three times. They also reported an increased Phosphorus-32 uptake in the femurs of lathyritic rats. The increased S^{35} uptake in the experimental animals was partly attributed by these authors to enhanced accrection of bone and cartilage that occurred secondary to slipping of muscular insertions on the femur. This is similar to the observation of Ponseti and Baird (41) that new bone formation occurred in metaphyseal region of long bones.

Ponseti, et al., in 1956 (61) did a study of the uptake of S^{35} in epiphyseal plates of lathyritic rats. The animals were made lathyritic by a 50% sweet pea diet and were all injected, as were the normal controls, with the same amount of radioactive sulphate per animal: 60 microcuries in 0.3 ml distilled water by the intraperitoneal route. All animals were sacrificed at 20 hours post-injection. These authors reported no difference in the gross intensity of the radioautographic image between the normals and lathyritic animals. All animals were given the same amount of S^{35} without regard for body weight differences.

Belanger in 1958 (48) injected subcutaneously one microcurie of S^{35} per gram of body weight into weanling rats which had been made lathyritic for ten to sixteen days by feeding diet of 50% ground Lathyrus odoratus peas and 50% regular chow.

He sacrificed the animals at two hours post-injection and prepared autoradiographs. His method consisted of comparing grossly the autoradiographs of lathyritic and normal controls, and he demonstrated 1) there was a greater accumulation of S^{35} in the diseased animals at the epiphyseal plate, 2) the epiphyseal plate was thicker, and 3) there was an increase in acellular ground-substance and also an increase in the number of chondrocytes over the normal animals. In younger animals he reported less plate hypertrophy, but the S^{35} still seemed to be greater in the lathyritic animals. He concluded that in the experimental animals there was an increase in the chondroitin sulphate at the epiphyseal plate. He also reported a decreased Calcium-45 binding in the epiphyseal plate matrix in vitro.

Kowalewski and Emery (33) in 1960 demonstrated a decreased uptake of S^{35} in the callus of healing fractures induced on lathyritic rats, and concluded that beta-aminopropionitrile inhibits bony repair.

Engfeldt, et al., (34) in 1960 demonstrated the findings of decreased S^{35} uptake after twenty-four and one-hundred-sixty-eight hours incubation in the epiphyseal plates of dogs given aminopropionitrile via stomach tube. They also reported a delayed transport of S^{35} from the chondrocytes to the cartilage matrix, as had been found to occur in rats (32, 69). However, these authors (34) found no differences in the metachromosia staining properties of the epiphyseal

cartilage and suggested increases in such staining properties in lathyrism are only seen in chronic or continuous cases of lathyrism.

Karnovsky and Karnovsky (58) in 1961 did quanatative uronic acid assays on isolated protamine salt of chondrotin sulphate and concluded that chondrotin sulphate was diminished in lathyritic epiphyseal cartilage. They noted increased metachromosia and alcian blue staining in lathyritic cartilage but concluded that this may not be due to purely an increase in mucopolysaccharides, but to an increase in the number of anionic groups available for binding the dyes. They performed a S^{35} uptake study by weighing and digesting the cartilage tissue and placing aluquots on planchets for counting in a "gas flow counter." The reason for the observed decreased uptake of S^{35} in lathyritic epiphyseal cartilage was not determined by these authors, but they postulated that it may be due to a loss of chrondroitin sulfate by diffusion secondary to decreased binding to chondromucoprotein, or that the loss could be due to depolymerization. They also noted the same decreased S^{35} uptake in <u>vitro</u> studies. Where the normal control animals gained weight at a greater rate than their experimental partners, the difference in S^{35} uptake was even more exaggerated. They observed that "depression of growth causes a nonspecific decrease in the Sulfur-35 incorporated into cartilage." They also reported hexos-amine levels unchanged in lathyrism as measured by

Carbon-14 uptake. They conclude with the warning that the study of S^{35} uptake is hard to evaluate because epiphyseal cartilage in weaning rats is not in a state of equilibrium, but has a rapid growth rate. The finding that there is no change in hexosamine content is in agreement with some (35) and in opposition to other findings (70).

Yuriko, et al., in 1962 (69) reported a decreased epiphyseal plate uptake of S^{35} given subcutaneously to lathyritic rats, after they had been on the lathyritic diet of beta-aminopropionitrile for 2, 3, 4, 5, and 6 weeks. They injected four microcuries per gram of body weight and sacrificed the animals at two hours, twenty-four hours, four and seven days later. The knee joints were utilized to prepare radioautographs. The intensity of the gross radioautographic image was reported less than the respective normal controls in every case.

In the lathyritic animals there was a delay in the movement of the S^{35} out from the chondrocytes to the cartilage matrix, and a further delay in movement from the matrix to the ossifying cartilage. When the lathyritic diet was changed back to normal there was an increased uptake noted in the epiphyseal plate, finally approaching the normal uptake when the rats had been off the diet for about eleven days. These authors conclude that there is a profound effect in lathyrism on the acid mucopolysaccharides of the epiphyseal cartilage matrix, but they are unable to delineate the metabolic site

of action.

Akamatsu, et al., (32) in 1964 did some studies on the uptake of S^{35} in the epiphyseal plates of lathyritic mice and concluded that mice are more resistant to lathyritic agents than are rats, as had been observed by other investigators (110), but the uptake of the radioactive sulphur is essentially the same as that of rats; that is, a decreased uptake in epiphyseal cartilage.

From carefully studied autoradiographs and other tissue methods, the uptake pattern of S^{35} in the epiphyseal cartilage and other tissues of the normal rat are well known (106, 66, 68, 108). In animals that have been made lathyritic the majority of investigators seem to have found a decreased S^{35} epiphyseal cartilage uptake (69, 32, 33, 34, 70). However, there are some who found that there is an increased uptake (39, 48) or no change either way (31, 61, 35). If we assume that most of the injected S^{35} is going to be incorporated into tissue chrondroitin sulphate as has been considered to be the case by many (105, 66, 67, 68, 107), then the frequent finding that there is a decreased S^{35} epiphyseal uptake in harmony with the decreased metachromosia noted by some (45, 111). However, other investigators have found an increased metachromosia (58, 53) in the epiphyseal cartilage, and perhaps this represents, as Karnovsky and Karnovsky (58) have said, not an actual increase in chrondroitin sulphate taking up the dye, but an increase in other

unknown substances having anionic groups available to bind the dye. In spite of conflicting findings, there is almost universal agreement that lathyrism profoundly affects the metabolism of mucopolysaccharides in growing rat cartilage, although the mechanism is not known.

A diligent search of the literature revealed no apparent report on the S^{35} uptake in non-epiphyseal lathyritic hyaline cartilage, such as that found in the xiphoid process of the rat.

Sulphur-35 uptake in lathyritic aorta

The first report found in the literature of an attempt to study the uptake of radioactive sulfur in the aorta of rats suffering from experimentally induced lathyrism was that of Ponseti, et al., in 1956 (61). This author induced lathyrism by feeding a 50% Lathyrus odoratus diet to five male rats beginning at 22 days of age. At 7 weeks of age, after having been on the diet for four weeks, those lathyritic animals, along with two normal controls, were each injected intraperitonealy with "approximately 60 microcurie of Sulfur-35" in the form of sodium sulphate. All animals were sacrificed twenty hours later and autographic emulsion sections were prepared and exposed for one week. These sections included femur, tibia, shoulder, and hip joints, thoracic and lumbar vertebrae, and thoracic aorta. The results showed the S^{35} to be "deposited in small amounts in the thoracic aorta of both control and experimental animals. No greater concentration

of S^{35} was observed in the aorta with the dissecting aneurysm." They did find an increased concentration of S^{35} uptake in the areas on long bones where periosteal detachments and increased bone formation had occurred in the lathyritic rats. They conclude that the uptake of S^{35} was the same in the chondroitin sulphate of the experimental and normal control animals and hence that the "sulphur content of the chondroitin sulphate is not greatly altered in lathyrism."

It is important to point out two aspects of the study reported by Ponseti et al.,: 1) all animals received the same amount of S^{35} without considering the probable differences in weight, and 2) this was not a time study extending over a period of several days but involved only the localization of S^{35} at twenty hours incubation.

Kowalewski in 1960 (36) did a study of S^{35} uptake in the aortas of cockerels made lathyritic by beginning a diet of 0.025% beta-aminopropionitrile at sixteen days of age. S^{35} in $H_2SO_4^{=}$, 1 microcurie per kg. of bird's body weight, was administered subcutaneously twenty-four hours before sacrifice. Sixteen percent of the birds died of dissecting aneurysms of the aorta. Weighed amounts of whole aortic tissue was analyzed for radioactivity, and the author reported no increased S^{35} uptake in the lathyritic aortas over normal controls.

It is important to point out in this study of Kowalewski's that although the concentration of S^{35} given was

based upon body weight, all animals were sacrificed at twenty hours post injection and hence we do not know how the uptake of S^{35} would change in respect to time.

The third and last report found in the literature on a study of the uptake of radioactive sulfur in the aorta of lathyritic animals was that of Kennedy and Kennedy in 1962 (31). These investigators induced lathyrism in rats by injecting beta-aminopropionitrile, amino-acetalnitrile, and by feeding a 50% diet of the sweet pea. They injected S^{35} in the form of $Na_2S^{35}O_4$, glycine labeled with Carbon-14, and Sulphur-35 DL-methionine by the intraperitoneal route in various of the lathyritic animals. They reported that the aortic radioautographs showed "no evidence of any significant difference in pattern or intensity of distribution." However, of significance is the fact that in none of the animals were they able to find any gross or microscopic changes in the aorta, and none of the animals died of dissecting aneurysm, although there were skeletal deformities. It would seem important to be able to demonstrate aortic lesions histologically before any valid conclusions could be drawn about abnormal patterns of aortic mucopolysaccharide metabolism as measured by S^{35}. For further discussion of the three studies (31, 36, 61) utilizing S^{35} aortic uptake in lathyrism refer to Chapter I - Introduction.

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